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(57) Abstract

The invention provides a two component system for the treatment of cancer comprising: (i) a tumour-directed antibody linked to a toxic agent or linked to an enzyme capable of converting a prodrug to a toxic agent; and (ii) an agent having the ability to restrict blood flow at the site of a tumour. Preferably the agent is a flavonoid derivative such as 5,6 dimethylxanthenone acetic acid or flavone acetic acid.

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CANCER THERAPY, USING ANTIBODY CONJUGATES, IN COMBINATION WITH A VASOACTIVE AGENT.

The present invention relates to novel combinations of compounds useful in the treatment of cancer, especially solid tumors, and to the use of such combinations in the treatment of cancers.

One long established approach to cancer therapy is the use of chemical agents which are toxic to cancer cells. A limitation on the use of all such chemotherapeutic agents is the toxicity they exert upon normal tissue. A continuing challenge to the art is the development of compounds which exert greater selectivity on tumor cells in comparison to normal tissue.

A second approach to fighting cancer which has been developed is the use of antibodies directed against tumour cell markers. Such antibodies may be linked to toxic agents so that when the antibodies bind to their tumour cell target, the toxic effect of the agent is exerted at the site of the tumour. Suitable toxic agents include toxic compounds such as ricin as well as radioisotopes, for example ^{131}I.

In a development of the above approach, antibodies have been linked to enzymes capable of activating a prodrug into an active drug, so that the antibody-enzyme conjugate can be delivered to a tumour site prior to administration to a patient of the prodrug, in order that the selectivity of the active drug can be enhanced, ie. the drug will only form at the site of the tumour. This antibody directed enzyme prodrug therapy (ADEPT) is disclosed in WO88/07378. A refinement of this system, in order to improve the localisation of the antibody-enzyme conjugate, is disclosed in WO89/10140.

Despite the advances mentioned above, there remains the need to improve the selectivity and activity of anti-cancer therapies.

We have now surprisingly found that tumour-directed antibodies linked to toxic agents have significantly increased activity when used in conjunction with a class of chemical agents which cause

haemorrhagic necrosis to occur at the site of a tumour. The increase in activity is greater than that which would be expected merely by the addition of the activity of the chemical agent and antibody alone.

Thus the present invention provides a two component system for the treatment of cancer comprising:

(i) a tumour-directed antibody linked to a toxic agent or linked to an enzyme capable of converting a prodrug to a toxic agent; and

(ii) an agent having the ability to restrict blood flow at the site of the tumour.

The invention further provides the above two component system for use in a method of treatment of the human or animal body, more especially for use in a method of treatment of tumours including solid tumours of the human or animal body.

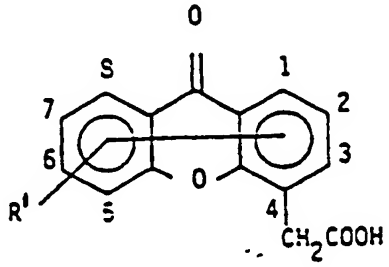
In a further aspect, the invention provides a method of treating cancer, including solid tumours, in a patient which comprises administering to the patient an effective amount of the two components of the invention. The two components may be administered simultaneously or sequentially in either order.

Tumours which may be treated include colon, lung (including small cell lung tumours), ovarian, breast, prostate tumours and lymphomas.

In another aspect, the invention provides a kit comprising the two components of the invention in a suitable container.

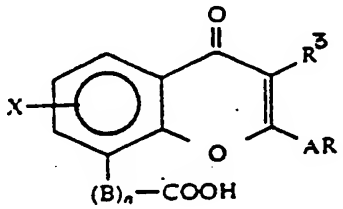
The agent having the ability to restrict blood flow to the tumour is preferably one which causes hemorrhagic necrosis. It will preferably be capable of inducing coagulopathy or haemorrhagic necrosis in the region of a tumour in the absence of the tumour-directed antibody.

Preferred agents having hemorrhagic activity are flavonoid compounds. Flavonoid compounds include xanthenone-4-acetic acids of the formula (I):



in which R^1 represents up to two groups which are independently selected from hydrogen, C_{1-6}, preferably C_{1-4} alkyl, F, Cl, Br, I, CF_3, CN, NO_2, NH_2, CH_2COOH, OR_2, OH, $NHCOR_2$, $NHSO_{2R2}$, SR_2, SO_2R_2, CH_2CONHR_2, or HNR_2 where R_2 is C_{1-6}, preferably C_{1-4} alkyl, or R^1 may represent the substitution of an asa (-N=) group for one or two of the methine (-CH=) groups in the carbocyclic rings or two of R^1 on any two available adjacent positions may also represent the grouping -CH=CH-CH=CH- to form an additional fused benzene ring; or a basic addition salt thereof. These compounds may be made by the methods disclosed in EP-A-0 278 176, the contents of which are incorporated herein by reference.

Other preferred flavonoid compounds include those of the formula (II):

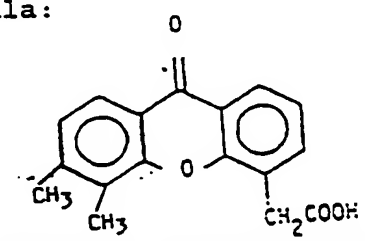


in which Ar is hydrogen, optionally substituted phenyl, thenyl, furyl, naphthyl, C_{1-6}, preferably C_{1-4} alkyl, C_{3-6} cycloalkyl or ara-C_{1-4} alkyl, B is a linear or branched C_{1-6} alkyene radical, either saturated or ethylinically unsaturated; R^3 is hydrogen or a phenyl radical, X is hydrogen or a C_{1-6}, preferably C_{1-4} alkyl or alkoxy group, and n=1, as well as salts, esters, aminoesters and amides thereof. These compounds may be made by the methods

disclosed in US-A-4,602,034, the contents of which are incorporated herein by reference.

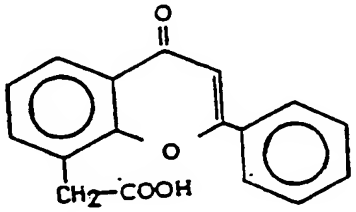
Further flavonoid compounds include those disclosed in US-A-5,116,954, the contents of which are incorporated herein by reference.

Flavonoid compound which are preferred include those of the formula (I) in which R^1 represents a single group selected from hydrogen, methyl eg. 2-methyl, 5-methyl or 6-methyl, ethyl, eg. 5-ethyl, methoxy, eg. 6-methoxy, ethoxy, eg. 5-ethoxy, or chloro, eg. 5-chloro or 6-chloro. Other compounds include those in which R^1 represents two groups, eg benzyl such as 5,6-dibenzyl. Especially preferred is the compound of formula (I) in which R^1 represents two methyl groups attached to the tricyclic ring at positions 5 and 6. This compound, 5,6-dimethylxanthenone acetic acid has the formula:



This compound is referred to below as dimethyl XAA.

A compound of the formula (II) which is especially preferred is [oxo-4-phenyl-2-4H-[1]-benzopyran-8-yl]acetic acid of the formula:



This compound is referred to below as flavone acetic acid or FAA.

Salts of the agent which may be conveniently used in therapy include physiologically acceptable base salts, eg derived from an appropriate base, such as alkali metal (e.g. sodium), alkaline earth metal (e.g. magnesium) salts, ammonium and NR_4 (wherein R is C_{1-4} alkyl) salts.

Further suitable agents may be obtained by assaying for compounds, including those of the above-mentioned references, which cause vascular disfunction at the site of tumours. For example, FAA has activity against subcutaneous solid mouse tumours and some activity against certain xenografts. It reduces blood flow and induces coagulopathy and haemorrhagic necrosis in the tumour as early as 4 hours after administration in mice with larger tumours experiencing more vascular dysfunction than small ones. Within 24 h up to 95% of the tumour may become necrotic and no tumour regrowth will occur from this region suggesting that cessation of blood flow following FAA is irreversible (Peters *et al*, 1991 Int. J. Radiat. Biol., 60;341-348). The mechanism of action is not only direct cytotoxicity as FAA is known to have immuno-modulatory activity. The vascular effects appear to be at least partially mediated by the induction of TNFα, as pre-treatment with anti-TNF antiserum prevents both blood-flow reduction and tumour regression produced by FAA in mice. Other compounds can be tested for a similar spectrum of activity and those which also have the required vascular necrotising activity can be used. Dimethyl XAA for example has been reported to have a 12-fold higher dose potency compared with FAA.

Other agents having the ability to restrict blood flow at the site of a tumour include vinblastine, hydralazine, midonisazole, tumour necrosis factor, or nitric oxide synthase inhibitors such as nitro-L-arginine.

The tumour-directed antibody may be an antibody specific for a tumour cell marker. We have used in our experiments the antibody A5B7 which is an anti-CEA (carcinoembryonic antigen) monoclonal antibody which recognises the human colon adenocarcinoma cell line LS174T (Pedley et al, 1987, Europ. J. Nucl. Med., 13; 197-202). However, many other tumour-cell-specific antibodies are known and may be used in the invention.

Examples of other antibodies include other antibodies against colon carcinoma (eg A5B7 or MFE-23 (Chester *et al*, 1994, Lancet 343;455-56)), as well as antibodies against small cell lung

carcinoma eg. anti-neural cell adhesion molecule antibody, ovarian cancer, lymphoma, breast cancer, prostate cancer (eg anti prostate specific antigen antibodies), non-small cell lung carcinoma (eg. anti CEA antibodies). Such antibodies are known in the art and can be obtained or made routinely.

The antibody used in the system of the invention may be monoclonal or polyclonal. For the purposes of this invention, the term "antibody", unless specified to the contrary, includes fragments of whole antibodies which retain their binding activity for a tumour target antigen. Such fragments include Fv, F(ab') and $F(ab')_2$ fragments, as well as single chain antibodies.

Furthermore, the antibodies and fragments thereof may be humanised antibodies, eg. as described in EP-A-239400.

The antibody may also be in the form of a fusion protein, with the antibody portion of such a protein being linked to another polypeptide which has a desired activity. For example, such a polypeptide may be tumour necrosis factor (TNF), in order to enhance the TNF activity produced by the agent having necrotic activity. Unless specified to the contrary, the term "antibody" includes such fusion proteins.

The antibodies may be produced by conventional hybridoma techniques or, in the case of modified antibodies or fragments, by recombinant DNA technology, eg by the expression in a suitable host vector of a DNA construct encoding the modified antibody or fragment operably linked to a promoter. Suitable host cells include bacterial (eg. E.coli), yeast, insect and mammalian.

The antibodies may also be produced by screening a recombinant DNA library, eg. a library made from cDNA from antibody producing cells. The library is screened with an antigen from a tumour cell which is to be targeted. Such techniques are described for example in McCafferty *et al* (Nature (1990) 348;552-54) or Chester *et al* (ibid). Unless specified to the contary antibodies obtained in this way, including single chain antibodies, are also covered by the term "antibody".

The antibody may be linked to a toxic agent. A toxic agent will be any agent capable of damaging or destroying a tumour cell to which the antibody has bound or in the environment of the cell to which the antibody has bound. This includes chemotherapeutic agents such as ricin or adriamycin and radioisotopes such as ^{125}I, ^{131}I, ^{67}Cu and ^{90}Y. Methods for linking such agents to antibodies are known in the art as such.

Alternatively (or in addition) the antibody may be linked to an enzyme capable of converting a prodrug to a toxic agent. Such antibodies may be produced and used in accordance with the techniques disclosed and described in WO88/07378. The antibody-enzyme conjugates may if desired be modified in accordance with the teaching of WO89/00427, in order to accelerate clearance from areas of the body not in the vicinity of a tumour. The antibody-enzyme conjugate may also be used in accordance with WO89/00427, eg, by providing an additional component which inactivates the enzyme in areas of the body not in the vicinity of the tumour.

The agent of the invention may be provided in the form of a pharmaceutical composition comprising the agent in combination with a pharmaceutically acceptable carrier or diluent, and optionally other therapeutic ingredients. The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipients thereof.

The formulations include those suitable for oral or parenteral (including subcutaneous, intramuscular, intravenous, intraperitoneal, intradermal, intrathecal and epidural) administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. Such methods include the step of bringing into association the active ingredient with the carrier which constitutes one or more accessory ingredients. In general the formulations are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers.

Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents, and liposomes or other microparticulate systems which are designed to target the compound to blood components or one or more organs. Such liposomes or other systems may themselves be linked to antibodies targeted to the tumour.

Suitable liquid carriers include phosphate buffered saline at a pH of between 7.0 and 8.0, for example 7.4. The formulations may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use.

The agent when formulated for oral administration may be presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution or a suspension in an aqueous liquid or a non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion.

Preferred unit dosage formulations are those containing a daily dose or unit, daily sub-dose, or an appropriate fraction thereof, of an active ingredient.

Although the dose of the antibody and agent according to the invention will ultimately be at the discretion of the physician, taking into account the nature of the condition being treated and the state of the patient, effective doses of agent may be in the range of from about 0.1 or 1.0 to 1000 mg/kg body weight, eg from about 20 to 200 mg/kg body weight, depending on the particular agent being used. For example, we have found that a suitable dose of FAA is about 200 mg/kg in both mice and humans whereas dimethyl XAA is more potent and active at about 20-30 eg 27.5

mg/kg in mice. The toxicity of any particular agent may be tested in the mouse xenograft model described in the examples below to determine the likely range of effective doses in humans.

The amount of antibody will depend upon the nature of the toxic agent or enzyme to which it is attached, although it should be similar to the amount of such an antibody which would be used in the art when treating tumours by the use of the antibody without the second component of the invention. This can be determined by reference to the prior art and/or by clinical experiment. By way of guidance, we have found that when the anitbody is linked to ^{131}I, a dose of about 10mg of antibody with an activity of 50-65 mCi/m^2 body surface area is suitable. However this dose is by way of guidance only and not limiting.

The antibody may be formulated as described above for the agent.

Both the antibody and agent may be administered by any suitable route of administration, eg parenteral (including subcutaneous, intramuscular, intravenous, intraperitoneal, intradermal, intrathecal and epidural) or oral.

In addition to the two components of the invention, other additional components may be used. For example, while not wishing to be bound by any particular theory, we believe that the tumour necrosis observed following the use of the system of the invention may be mediated at least in part by tumour necrosis factor (TNF). Thus, TNF may also be administered to patients undergoing treatment with the system of the invention. In such a case, the TNF may be administered before, with or after administration of either component of the invention although desirably it will be administered following localization of the antibody component. Doses of TNF and routes of administration may be determined by reference to those of skill in the art. A preferred formulation of TNF for administration is in the form of a liposome formulation, since liposomes will become lodged in the tumour and taken out of circulation more quickly than free TNF. Such liposomes may themselves be linked to antibodies targeted to the tumour.

Other agents which may be administered with the system of the invention include conventional anti-cancer drugs such as doxorubicin or 5-fluorouracil.

When the system of the invention is administered to a patient for the treatment of a tumour, although the two components may be administered together or sequentially in either order, it is preferred that the antibody component of the invention is administered prior to administration of the haemorrhagic agent component. Preferably, sufficient time is allowed to elapse following administration of the antibody component for the antibody to localise before the haemorrhagic agent is administered. The rate at which the antibody component localises may be monitored by labelling a proportion of the antibody administered to the patient with a suitable imaging label, eg. ^{131}I, ^{99m}Tc or ^{111}In. The monitoring will help determine the optimum time, although a time of from about 3 to about 48, eg about 12 to 24 hours may be suitable.

The following examples illustrate the invention.

Example 1

MATERIALS AND METHODS

Drugs

Flavone acetic acid (FAA) was made up in saline and given intraperitoneally (ip) at a dose of 200mg/kg, which is within the dose range given to patients in clinical trials with this drug.

Antibody labelling

A5B7, a monoclonal anti-CEA antibody (Pedley *et al.* 1976 Europ. J. Nucl. Med. 13; 197-202), was labelled with ^{131}iodine by the chloramine T method to a specific activity of approximately 10μCi/μg protein, and sterilised by passing through a 0.22mm Gelman filter (Northampton, UK).

Animal studies

A human colon adenocarcinoma cell line LS174T (Tom *et al*, 1976 *In Vitro*, 12; 180-181) was used to develop a xenograft model in female nude (nu/nu) mice by subcutaneous inoculation into the flank. Subsequent passaging was by continuous subcutaneous implantation from the original xenograft. The tumour is a moderately differentiated CEA-producing adenocarcinoma which secretes no measurable CEA into the circulation. The A5B7 gives positive staining for glandular luminal surface and cytoplasm. All mice used were 2 to 3 months old, and weighed between 20-25g at the initiation of experiments.

Radioimmunotherapy studies

The experiments proceeded when the tumours reached 0.1-0.2cm^3 and were in exponential growth, approximately 10 to 14 days after passaging, using 6 mice per group. The radiolabelled antibody was administered via the tail vein as a single injection of 0.5mCi/50μg per mouse. One group of animals received a single dose of FAA (200mg/kg) at either 24 or 48 hours after radioantibody administration, thus allowing time for tumour localisation of the antibody. Additional groups received either FAA alone (200mg/kg) or no treatment. Tumours were measured and the mice weighed on the day of antibody injection and on every subsequent 3rd or 4th day until tumour volume reached 2cm^3 when the mice were culled. The measurements were carried out in 3 dimensions (L, W & H), and the volume estimated as LWH/2.

Effect of FAA on biodistribution

In order to assess the effect of FAA on antibody distribution, the ^{131}I A5B7 was administered intravenously at a dose of 50μCi/5μg, using 4 mice per group. FAA at a dose of 200mg/kg was injected intraperitoneally at the following times in relation to antibody administration: 3h before, at the same time, 3h after and 24h after antibody. The animals were bled and culled at selected time points and tumour, liver, kidney, lung, spleen, colon and muscle removed for activity assessment on the gamma counter (Wizard, Pharmacia). Mice were given food and water ad libitum, the water containing 0.1% potassium iodide during experiments to order to block thyroid uptake of iodide.

Histological Studies

Tumour tissue from test and control animals of the different biodistribution groups was examined histologically in sections stained with haematoxlin and eosin in order to investigate the effect of FAA administration on tumour structure. In addition, the microdistribution of the antibody was studied by autoradiogrphy.

RESULTS

Effect of FAA on tumour morphology

By 4h after administration of FAA there was evidence of haemorrhage in the centre of the tumour, with distension of some of the smaller vessels at the periphery. By 24h, extensive haemorrhagic necrosis could be seen throughout the tumour, with some nodules showing evidence of a thin peripheral rim of viable tumour cells.

Effect of FAA on radioimmunotherapy

Figure 1 shows the mean tumour growth following either 0.5mCi ^{131}IA5B7 alone or when combined with FAA, administered 24h after radioantibody. Both these treatments produced a significant therapeutic effect when compared to the control group, but the addition of FAA following radioantibody produced a further significant inhibition of tumour growth and prolongation of survival. For mice receiving radioantibody alone, the mean tumour volume started to increase at 24 days after antibody injection, and by 80 days all tumours in the group had exceeded 2cm^3. For the group of mice receiving combined radioantibody and FAA treatment, the mean tumour size did not exceed the initial volume until 60 days after antibody injection, and at 120 days three of the tumours are too small to measure. Similar results were obtained when the administration of FAA was delayed until 48h after giving the ^{131}I-A5B7 (Figure 2). While the tumours of all 6 mice receiving ^{131}I-A5B7 had commenced a slow regrowth by 24 days after antibody administration, those of only 2 mice receiving a concomitant dose of FAA had commenced regrowth by 86 days. All control mice had been culled by 21 days after initiation of the experiment, when their tumours exceeded 2cm^3.

Administration of FAA alone inhibited tumour growth for a few days only, and produced no significant prolongation of survival when compared to the control mice (Figure 2).

Effect of FAA on biodistribution of radioantibody

The biodistribution and clearance of ^{131}I-A5B7 was studied, with or without FAA given at 48h post antibody administration. No difference between groups was seen in antibody localisation to tumour or normal tissues at 4h, 24h or 72h after FAA administration. However, by 120h post FAA (168h post radioantibody) there was significantly greater tumour localisation in the group receiving FAA than in those without, suggesting that the antibody was trapped within the necrosis caused by the drug (Figure 3).

Example 2

Six mice were treated with 0.5 mCi ^{131}I-A5B7 antibody following the introduction of the LS174T xenograft model as described above. A further 6 were treated with the labelled antibody and 30 mg/kg dimethyl XAA administered as described for FAA. Two groups of 6 mice were used as controls and given either dimethyl XAA alone or were untreated.

Figure 4 shows the mean tumour growth following either 0.5mCi ^{131}I-A5B7 alone or in combination with 30 mg/kg XAA, administered 48 hrs. after the radioantibody. As in the case of FAA, combined administration of radioantibody and XAA significantly enhanced both the length of tumour-growth inhibition and survival time over that found for ^{131}I-A5B7 alone. Some toxicity was seen at this drug dose, 2 mice of 6 from both the combined therapy group and those receiving XAA alone dying within 2 days of treatment. However, while the tumours of all mice in the RIT group alone had exceeded 2cm^3 none of the 4 mice remaining in the combined therapy group had a measurable tumour after 6 months, and showed no long-term toxicity effects. XAA alone did not prolong survival when compared with the control group, although tumour necrosis was again produced.

Example 3

A range of concentrations of dimethy XAA were administered intraperitoneally to groups of 2 mice with tumours, and the tumours removed 72 hours later for histological examination.

At 20 mg/kg, only 1 of the 2 tumours showed a small area of necrosis, the majority being viable tissue. there was no evidence of enhanced radioimmunotherapy when combined with XAA at this dose.

At 27.5 and 30 mg/kg there was massive haemorrhagic necrosis, with only a thin viable rim of peripheral tumour cells remaining. Both these doses show significant enhancement of radioimmunotherapy when given with 0.5 mCi ^{131}I-A5B7. Figure 5 shows the results for 27.5 mg/kg.

Example 4

FAA administered to humans is known to be safe, although some minimal side effects at a dose of 4.8 g/m^2 given over one hour were observed. The most common side effects were a feeling of warmth during infusion, transient nausea and blurring of vision (Havlin *et al*, (1991) J. Natl. Cancer Inst., 83; 124-128).

A clinical trial was instituted at the Royal Free Hospital, London, UK. Patients with refractory or relapsed colorectal cancer, who meet the inclusion criteria, entering the trial are given an intravenous infusion of 10-15 mg A5B7 F(ab')2 (murine anti-CEA antibody) conjugated with 2035MBq/m^2 of ^{131}I over 30 mins. 6 hours later an intravenous infusion of 4.8 g/m^2 in 500 mls of 0.9% saline of FAA is given. Immunosuppressive agents, thyroid blocking agents and appropriate pre and post hydration are alos given. Blood is collected for ^{131}I and TNF assay. Serial γ camera images are collected to assess antibody distribution and perform dosimetric calculations on tumour, liver, lung and kidney at time-points outlined in the protocol (4hrs, 24hrs, 48hrs, 72hrs, 10 days). Terminal elimination half-lives are calculated for tumour, normal organs and blood for comparison to those determined in previous radio-immunotherapy trials undertaken without FAA. Patients can be treated up to 4 times depending on

response, toxicity and the development of an immune response to A5B7 F(ab')2.

By April 1994 6 (of a projected 10) patients had been entered into the trial and received a total of 8 treatments. Median follow up is at present 2 months. The administration of the antibody has been uncomplicated. The administration of the FAA infusion has been complicated by transient diarrhoea, hypertension and visual disturbances. Post treatment toxicity has been graded according to EORTC Common Toxicity Criteria.

CLAIMS

1. A two component system for the treatment of cancer comprising:
   (i) a tumour-directed antibody linked to a toxic agent or linked to an enzyme capable of converting a prodrug to a toxic agent; and
   (ii) an agent having the ability to restrict blood flow at the site of a tumour.

2. A system according to claim 1 wherein the agent causes haemorrhagic necrosis and is a flavonoid derivative.

3. A system according to claim 2 wherein the flavonoid is 5,6-dimethylxanthenone acetic acid or a salt thereof, or [oxo-4-phenyl-2-4H-[1]-benzopyran-8-yl]acetic acid or a salt thereof.

4. A system according to any one of the preceding claims wherein the antibody is an antibody against a tumour cell marker.

5. A system according to claim 4 wherein the tumour cell marker is specific for breast cancer or colon cancer cells.

6. A system according to any one of the preceding claims wherein the antibody is a humanised antibody.

7. A system according to any one of the preceding claims wherein the antibody is an antibody fragment which retains its binding activity against its target.

8. A system according to any one of the preceding claims wherein the antibody is linked to a toxic agent.

9. A system according to claim 8 wherein the toxic agent is ricin, adriamycin or ^{131}I.

10. A system according to any one of the preceding claims in which the antibody is linked to an enzyme capable of converting a prodrug to a toxic agent.

11. A kit which comprises:

(i) an antibody linked to a toxic agent or linked to an enzyme capable of converting a prodrug to a toxic agent as defined in any one of the preceding claims in association with a pharmaceutically acceptable carrier or diluent; and

(ii) an agent as defined in any one of the preceding claims in association with a pharmaceutically acceptable carrier or diluent.

12. A system according to any one of claims 1 to 10 or a kit according to claim 11 for use in a method of treatment of the human or animal body.

13. A method of treating cancer in a patient which comprises administering to the patient, an effective amount of the two components system as defined in any one of claims 1 to 10 or a kit as defined in claim 11.

14. A method according to claim 13 wherein the antibody component of the system is administered prior to administration of the agent.

Fig. 1.

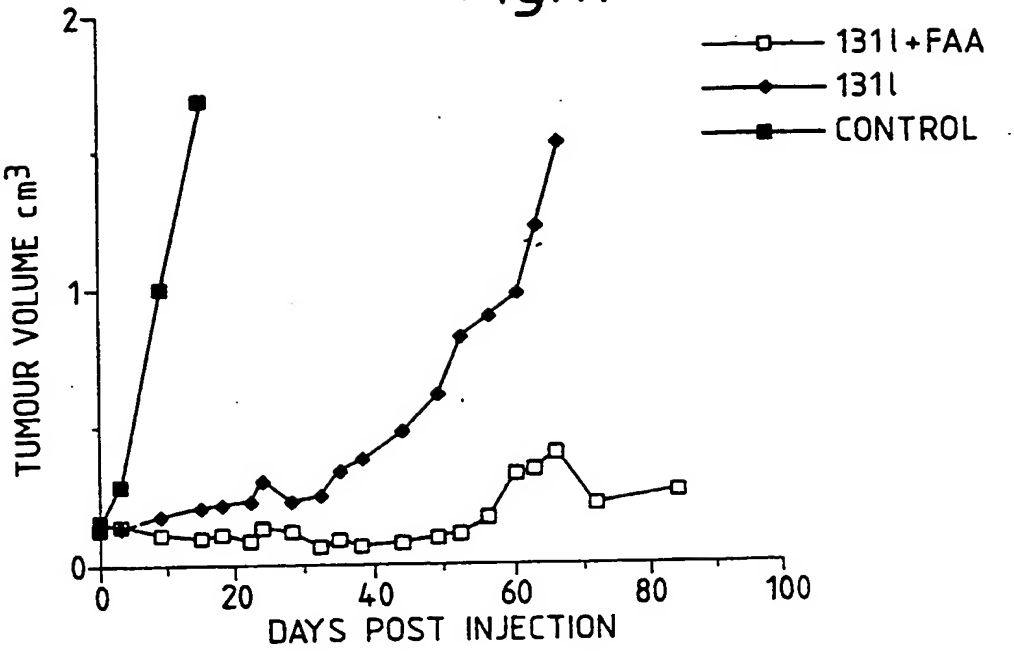

131I+FAA
131I
CONTROL
TUMOUR VOLUME cm3
2
1
0
0
20
40
60
80
100
DAYS POST INJECTION

Fig. 2.

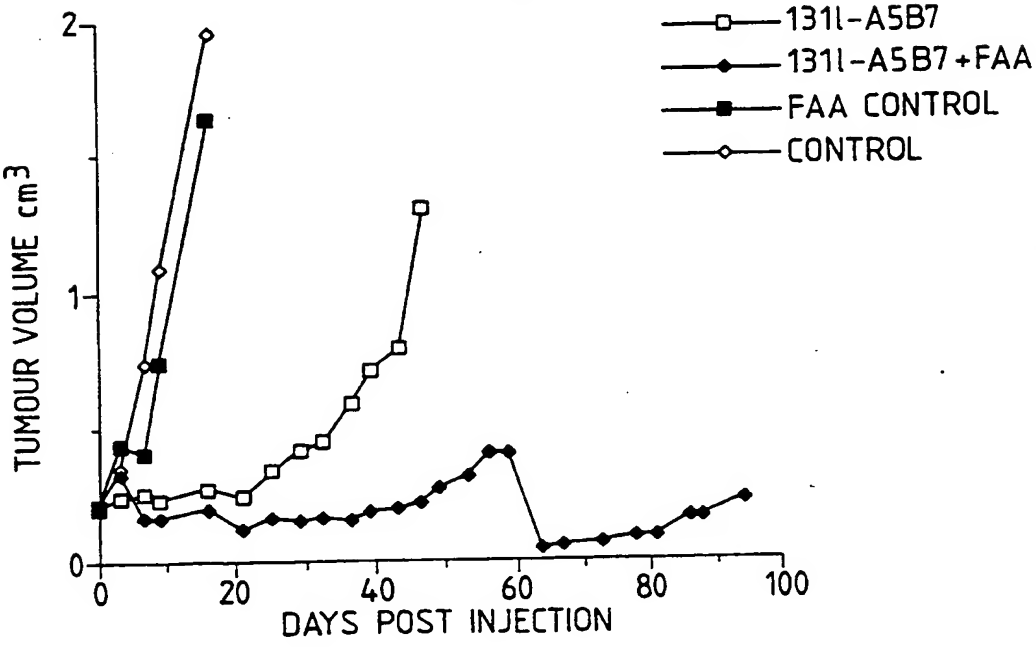

131I-A5B7
131I-A5B7+FAA
FAA CONTROL
CONTROL
TUMOUR VOLUME cm3
2
1
0
0
20
40
60
80
100
DAYS POST INJECTION

Fig. 3.

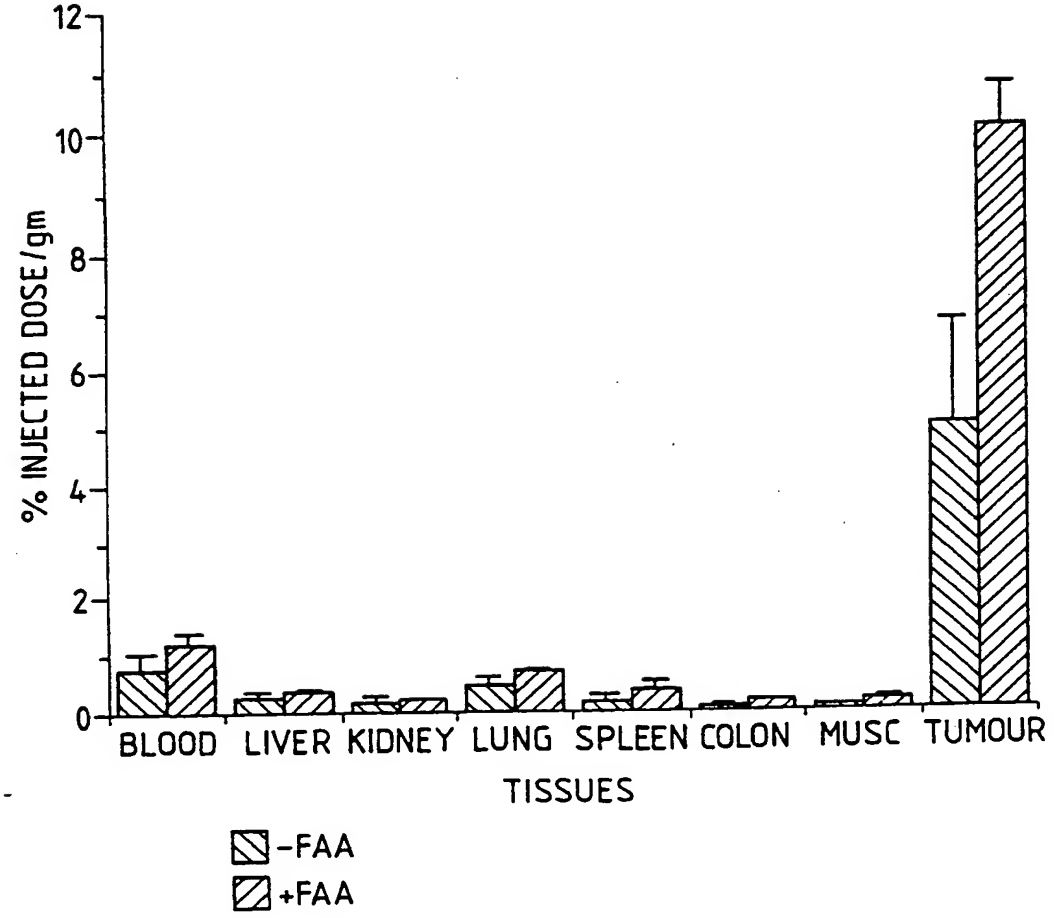

12
10
8
6
4
2
0
% INJECTED DOSE/gm
BLOOD
LIVER
KIDNEY
LUNG
SPLEEN
COLON
MUSC
TUMOUR
TISSUES
-FAA
+FAA

Fig.4.

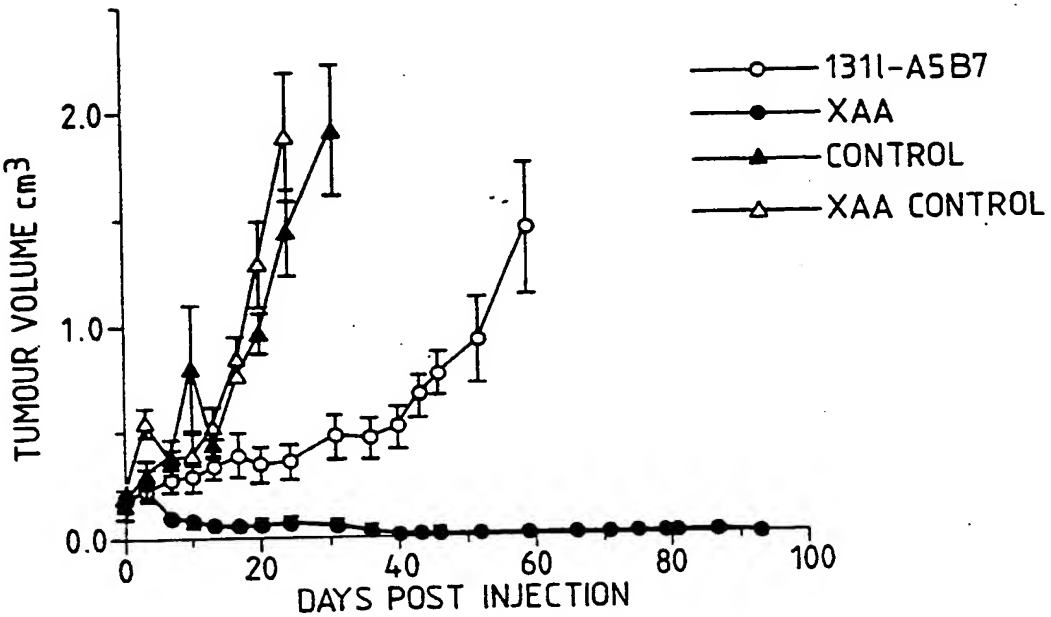
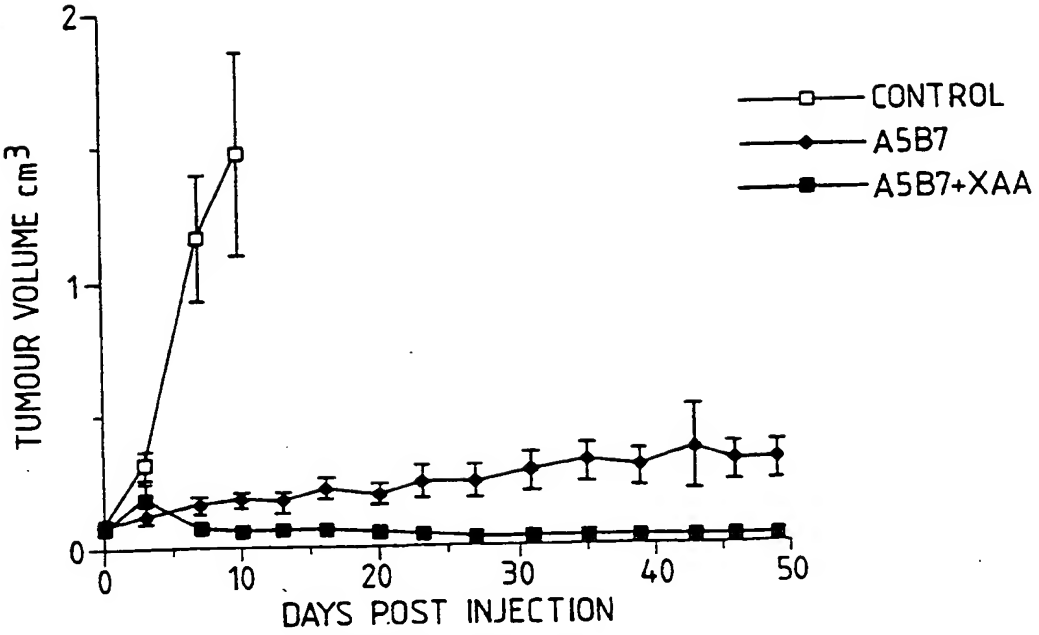

131I-A5B7
XAA
CONTROL
XAA CONTROL
TUMOUR VOLUME cm3
2.0
1.0
0.0
0
20
40
60
80
100
DAYS POST INJECTION

Fig.5.


CONTROL
A5B7
A5B7+XAA
TUMOUR VOLUME cm3
2
1
0
0
10
20
30
40
50
DAYS POST INJECTION

INTERNATIONAL SEARCH REPORT

In tional Application No
PCT/GB 94/00831

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 A61K47/48 A61K31/35

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	CANCER CELLS (US), MARCH 1991, VOL. 3, NO. 3, PAGE(S) 100-2, Moore JV et al 'Vasculature as a target for anti-cancer therapy.' see page 101, middle column - page 102 ---	1-14
Y	INT. J. RADIAT. BIOL., 1991, VOL. 60, NOS. 1/2, PAGE(S) 395-9 Bibby, M. C. et al 'Flavone acetic acid: is vascular shutdown the crucial mechanism of action?' see page 396 - page 398 --- -/--	1-14

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 August 1994	13.09.94

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Dullaart, A

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	BR. J. CANCER, 1990, VOL. 62, NO. 2, PAGE(S) 231-7 Zwi, L. J. et al 'The use of vascularized spheroids to investigate the action of flavone acetic acid on tumor blood vessels' see page 233 see page 236	1-14
Y	EUR J CANCER (GB), 1991, VOL. 27, NO. 6, PAGE(S) 765-70, Murray JC et al 'Flavone acetic acid potentiates the induction of endothelial procoagulant activity by tumour necrosis factor.' see Discussion	1-14
Y	PROC ANNU MEET AM ASSOC CANCER RES, VOL. 29, ABSTRACT NO. 1619, 1988 Hornung RL et al 'MECHANISM(S) BY WHICH FLAVONE ACETIC ACID AUGMENTS NK ACTIVITY AND SYNERGIZES WITH RIL2 FOR TREATMENT OF MURINE RENAL CANCER (MEETING ABSTRACT)' see abstract	1-14
Y	WORLD J. UROL. (DE), 1991, VOL. 9, NO. 4, PAGE(S) 192-197, Van Moorselaar R.J.A. et al 'Use of animal models in diagnosis and treatment of renal cell carcinoma. An overview.' see page 195 - page 196	1-14
Y	EUR. J. NUCL. MED., 1987, VOL. 13, PAGE(S) 197-202. PEDLEY ET AL. 'Relationship between tumour size and uptake of radiolabeled anti-CEA in a colon tumour xenograft' cited in the application see Discussion	1-14
Y	WO,A,88 07378 (CANCER RESEARCH CAMPAIGN TECHNOLOGY LTD.) 6 October 1988 cited in the application see the whole document	1-14
Y	WO,A,89 10140 (CANCER RESEARCH CAMPAIGN TECHNOLOGY LTD.) 2 November 1989 cited in the application see the whole document	1-14

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INTERNATIONAL SEARCH REPORT

Int ional Application No
PCT/GB 94/00831

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EUR J CANCER (GB), 1993, VOL. 29A, NO. 5, PAGE(S) 729-33, Chabot GG et al 'Tumour necrosis factor-alpha plasma levels after flavone acetic acid administration in man and mouse.' see page 729	1-14
T	INT J CANCER (US), 15 JUNE 1994, VOL. 57, NO. 6, PAGE(S) 830-5, Pedley RB et al 'Enhancement of radioimmunotherapy by drugs modifying tumour blood flow in a colonic xenograft model.' see the whole document	1-14

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB94/00831

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 13, 14
because they relate to subject matter not required to be searched by this Authority, namely:
REMARK: ALTHOUGH CLAIM 13 AND 14 ARE DIRECTED TO A METHOD OF TREATMENT OF THE HUMAN OR ANIMAL BODY (PCT RULE 39.1 (iv)), THE SEARCH HAS BEEN CARRIED OUT BASED ON THE ALLEGED EFFECTS OF THE COMPOUND/COMPOSITION.

2. [X] Claims Nos.: 1- 14
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
IN VIEW OF THE LARGE NUMBER OF COMPOUNDS, WHICH ARE DEFINED BY THE GENERAL DEFINITIONS OF BOTH COMPONENTS TO BE USED, THE SEARCH HAD TO BE RESTRICTED FOR ECONOMIC REASONS.
FOR FURTHER INFORMATION SEE ANNEX

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

CONTINUATION OF BOX I.2

THE SEARCH WAS LIMITED TO THE COMPOUNDS FOR WHICH PHARMACOLOGICAL DATA WAS GIVEN AND/OR THE COMPOUNDS MENTIONED IN THE CLAIMS, AND TO THE GENERAL IDEA UNDERLYING THE APPLICATION (SEE GUIDELINES, PART B, CHAPTER III, PARAGRAPH 3.6).

INTERNATIONAL SEARCH REPORT

Information on patent family members

Ir ational Application No
PCT/GB 94/00831

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-8807378	06-10-88	DE-D-	3889340	01-06-94
		EP-A-	0408546	23-01-91
WO-A-8910140	02-11-89	EP-A-	0414741	06-03-91
		JP-T-	3503898	29-08-91